Abstract

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Title of diploma thesis: Study on possible utilization of metal oxide based stationary phases for the analysis of polar compounds

This diploma thesis is aimed at investigation of the ability of zirconia (column ZirChrom®-PHASE, 150 x 4,6 mm; 5 μm) and titania (column Sachtopore®-NP, 150 x 4,6 mm; 5 μm) based stationary phases for analyses under the conditions of hydrophilic interaction chromatography (HILIC).

Influence of mobile phase strength, mobile phase pH and buffer strength on the separation of biologically important polar compounds: adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), cyclic adenosine monophosphate (cAMP), creatine (C) and phosphocreatine (CP) was tested.

All analyses were performed using isocratic elution. The mobile phases consisted of an organic part – acetonitrile (ACN) and an aqueous part – buffer (sodium phosphate dibasic – Na_2HPO_4 with sodium fluoride – NaF) in different ratios. The mobile phase flow rate was at 1 mL/min. The detection of analytes was performed by using a photodiode array detector at wavelengths of 215 nm for C and CP and 254 nm for AMP, ADP, ATP and cAMP.

The retention of the analytes as well as column efficiency increased with increasing proportion of an organic component in the mobile phase. The column efficiency was also improved using higher mobile phase pH. A higher concentration of sodium fluoride in a buffer resulted in to better peak shape and symmetry. A very good separation of all analytes was achieved on both columns using the mobile phase composed of 67 % ACN and 33 % buffer (10 mM Na_2HPO_4 and 5 mM NaF) at pH 7.